TRANSLATION

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
H 10020 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/012931	15.11.2004	14.11.2003
International Patent Classification (IPC) or national classification and IPC		
C12N7/00		
Applicant		
HOLM, Per Sonne		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 11 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *(sent to the applicant and to the International Bureau)* a total of _____ sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____ , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
Name and mailing address of the IPEA/EP	Authorized officer
Facsimile No.	Telephone No.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/012931

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (Rule 12.3 and 23.1(b))

☐ publication of the international application (Rule 12.4)

☐ international preliminary examination (Rule 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☐ the international application as originally filed/furnished

☒ the description:

pages 1-123 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

nos. 1-103 as originally filed/furnished

nos.* _____ as amended (together with any statement) under Article 19

nos.* _____ received by this Authority on _____

nos.* _____ received by this Authority on _____

☒ the drawings:

sheets 1/33-33/33 as originally filed/furnished

sheets* _____ received by this Authority on _____

sheets* _____ received by this Authority on _____

☒ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☐ the claims, nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

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Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-103	YES
	Claims		NO
Inventive step (IS)	Claims		YES
	Claims	1-103	NO
Industrial applicability (IA)	Claims	1-103	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

V.1 This report makes reference to the following documents:

D1: DE 101 50 945 (Holm, P.S.)

D2: US 2002/0086411 (Holm, P.S.)

D3: Holm, P.S. et al. (2002) YB-1 relocates to the nucleus in adenovirus-infected cells and facilitates viral replication by inducing E2 gene expression through the E2 late promoter. JBC 277, 10427 - 10434.

V.2 **Novelty** (PCT Article 33(1) and (2))

V.2.1. Prior art:

D1 proposes improvements of the virally inducted oncolysis of known adenoviral systems, for example through deletions of specific early genes.

D2 describes E1A-deficient adenoviruses (Ad) that were produced proceeding from the discovery that E1A-deficient adenoviruses are capable of replicating [0020] in tumor cells that have YB-1 in the nucleus, and these adenoviruses have a DNA sequence that codes for YB-1.

D3 discloses a new function of the E1B-55kDA protein for adenoviral replication, that is, the facilitated accumulation of the YB-1 protein in the cell nucleus that occurs in conjunction with the E2 transcription. Furthermore, the specific interaction of YB-1 with the promoter of the proximal Y box of the late E2 promoter is shown as well as the fact that YB-1 controls the activity of the late E2 promoter.

V.2.2 Irrespective of the observations below with respect to the clarity of claim 1, the subject matter of said claim is regarded as novel within the meaning of PCT Article 33(1) and (2) because none of the cited documents discloses a change in the order of the E1A or E1B and/or E4 gene expression.

V.3 **Inventive step** (PCT Article 33(1) and (3))

V.3.1 D2, which is regarded as the closest prior art, discloses E1A-deficient adenoviruses that contain a DNA sequence that codes for

YB-1 and are capable of replicating in tumor cells having YB-1 in the nucleus.

V.3.2 The subject matter of claim 1 differs therefrom in that the adenovirus expresses a protein selected from the group that includes an EA1 protein, and, in fact, carries out said expression after the expression of a first protein selected from the group that includes an E1B protein and an E4 protein.

V.3.3 The effect obtained thereby is described as follows in the present application:
"This does not occur, however, in the E1A-minus adenoviruses known from the prior art, in which the 13S E1A protein is not present. The nucleus localization of YB-1 in multidrug rsistent cells having YB-1 in the nucleus allows the replication or particle formation of such E1A-minus viruses. In such a case, however, the efficiency of the viral replication or particle formation is significantly reduced compared to that of the wild type Ad5. A combination of YB-1 which either is already present in the nucleus of the tumor cell, whether because YB-1 is present in the cell nucleus independently of the cell cycle, or whether because deregulated YB-1 present in the cytoplasma is translocated into the cell nucleus by the adenoviruses of group I and/or II, or is induced into the cell nucleus, i.e. prompted

to be present in the cell nucleus, or is introduced as a transgene by a vector, with a system, preferably with an adenoviral system that activates the adenoviral genes but is not capable of viral replication, surprisingly results, in comparison, in a system that allows very effective viral replication or particle formation promoted by YB-1, and therefore an oncolysis. The same also applies to the claimed adenoviruses, i.e. the adenoviruses of group I, which, owing to their specific design and utilization of the effect that an E1B protein, and preferably the E1BSSK protein and/or an E4 protein, preferably the E4orf6 protein, are suitable for effectively providing YB-1, preferably in the nucleus, for effective replication (page 59, lines 1-24).

V.3.4 The problem to be solved by the present application can therefore be regarded as that of providing an adenovirus for improved oncolysis, i.e. one that makes possible an effective viral replication or particle formation.

V.3.5 The solution to the problem as proposed by the present application, i.e. the "adenovirus, which expresses a first protein selected from the group that includes an E1B protein and an E4 protein, before a second

protein selected from the group that includes and E1A protein" cannot currently be regarded as inventive within the meaning of PCT Article 33(1) and (3), since the above-mentioned effect is not supported by the description and the problem is consequently not regarded as solved:

Although the application discloses two embodiments of claim 1, namely the adenoviruses XvirPSJL1 and XvirPSL2 (figures 21 and 22), the technical effect described (page 59, lines 1-24), which involves an improvement over the E1A-minus viruses known from the prior art, is not demonstrated through experimental data. The description merely discloses the production of these constructs (example 16). Examples 14, 15 and 17, which show that deregulated YB-1 can be transported into the nucleus using E4orf6 and E1B-55K — and also without E1A - and induces the E2 gene expression there, hearken back to the adenoviruses Ad312, Ad520 and Δ24, which are known from the prior art.

V.3.5.1 For the same reasons, claims 54, 55, 59, 62, 88 and 103 are likewise regarded as failing to involve an inventive step.

V.3.5.2 Since the dependent claims also fail to contain any technical feature that, either alone or in combination with any one of the

claims to which it refers or which appears to involve an inventive step, they do not meet the requirements of PCT Article 33(1) and (3).

V.3.5.3 Consequently, claims 1-103 do not meet the requirements of PCT Article 33(1), since they cannot currently be regarded as involving an inventive step.

V.4 **Industrial applicability** (PCT Article 33(1))

V.4.1 The subject matter of claims 1-103 appears to meet the requirements of PCT Article 33(1) with respect to industrial applicability.

Box No. VIII **Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

VIII.1 **Completeness of the disclosure** (PCT Article 5) and **clarity** (PCT Article 6)

VIII.1.1 Claim 1 of the present application does not meet the requirements of PCT Article 6, because the subject matter for which protection is sought is not clearly defined. It attempts to define the subject matter of the claim in terms of the result to be achieved, i.e. the expression of a first protein before the expression of a second protein. Such a definition cannot be regarded as clear within the meaning of PCT Article 6, since it does not refer to any technical features that would prompt a person skilled in the art to reproduce the claimed adenovirus without unreasonable difficulty. The present application discloses two embodiments of the adenovirus indicated in claim 1 that are shown in figures 21 and 22 and contain enough technical features to characterize the subject matter of claim 1 in greater detail. This is also apparent from the wording of the claims dependent on claim 1, since they make reference to said features. Therefore, the present wording of claim 1 cannot be regarded as the only possible wording. As a result, these technical features have to be included in the wording for the characterization of the

Box No. VIII **Certain observations on the international application**

subject matter of claim 1 in order to meet the requirements of PCT Article 6. Furthermore, the present form is not supported throughout the entire scope for which protection is sought: the description indicates that it is not possible to use every EA1, EB1 and/or E4 protein to achieve the desired effect. Consequently, the claim in its present from does not meet the requirements of either PCT Article 5 or PCT Article 6.

Moreover, it should be noted that the current version of this claim does not indicate clearly that "before" refers only to the temporal order of the gene expression or to how it can be achieved.

VIII.1.2 For the same reasons, claims 63-87 and 89-102 do not appear to be supported by the description and therefore they do not meet the requirements of PCT Article 6.

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment* on _____

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

The sequence listing in the description, pages 1-9, received by this authority on 14.06.2005 with letter of 13.06.2004.

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*